Date: 03/3/14 People in lab: Levi Palmer, Kira Buckowing

Title: Genome prep of P aeru

Start Time: 4:00 PM

Purpose: To purify the genomic DNA of P. aeru to improve PCR results

Protocol: MN DNA, RNA purification kit protocol for genome prep

Products:

Sample Label	Description	Quantity
P.aeru 1 3/3	Genome prep of P. aeruginosa	2

Results: 1: Conc. 88.9 ng/uL, 260/280: 1.5, 230/260: 1.99 2. Conc. 105.4 ng/uL, 260/280: 1.68, 230/260: 2.23

Notes:

Stop Time: 7:00 PM

Next: PCR amplify norV as control and norCB

Date: 03/06/14 People in lab: Levi Palmer

Title: Miniprep of hmp from 2/25 TOPO

Start Time: 12:00 PM

Purpose: To purify the hmp plasmid from the transformed cells to test for correct part by digest

Protocol: LTM ed. 2

Products:

Sample Label	Description	Source Label	Quantity
Poss hmp 3/6	Purified DNA from possible hmp	Poss. hmp 2/25	1

Results: 1: Conc. 45 ng/uL, 280/260: 2, 230/260: 2

Notes:

Stop Time: 4:00PM

Next: Digest to check for hmp gene by electrophoresis

Date: 03/10/14 People in lab: Levi Palmer

Title: Digest of hmp from 3/6/14

Start Time: 5:00PM

Purpose: To cut out the hmp gene, if present, from the TOPO vector, to be confirmed by electrophoresis

Protocol: LTM ed 2

Products:

Sample Label	Description	Source Label	Quantity
D1 3/10 hmp LP	digested TOPO vector with hmp possibly	Poss hmp 3/6	1

Stop Time: 7:00 PM

Next: Gel electrophoresis to look for hmp fragment

Date: 03/11/14 People in lab: Levi Palmer

Title: Gel electrophoresis of 3/10 Digest

Start Time: 11:00 AM

Purpose: To check for presence of hmp gene in digest and TOPO vector

Protocol: LTM ed. 2

Results: No hmp present, no bands present, no picture taken

Next: Retry hmp PCR and TOPO cloning

Date: 03/11/14 People in lab: Levi Palmer

Title: Making chemically competent cells

Purpose: To make chemical competent cells to use in lab.

Protocol: OpenWetWare: Making Chemically Competent Cells (Inoue) **Exceptions:** 1. O.D. <0.1 of SOB grown culture 2. Adjusted to volume of 250mL 3. 1/2 volume of final resuspension

Products:

Sample Label	Description	Source Label
Chemical Competent 3/11/14	Chemically competent DH5alpha, in -80C	DH5alpha stock

Notes: Test for competence was successful.

Date: 03/11/14 People in lab: Levi Palmer

Title: PCR of norCB, nosZ, hmp, norV from Genome Prep and E. coli colony PCR, then gel electrophoresis to confirm

Purpose: To amplify the genes and check for successful PCR, also a check for successful genome prep.

Protocol: LTM ed. 2 pg 48, 44-46

Results: No bands visible for norV, norCB, nosZ. Strong band visible for hmp

Well	1	2	3	4	5	6	7	8
Sample	norV	hmp	norCB		Ladder	RGTT		LMPG

Notes: The wrong organism was used for norV, that is why there was no product. norV is an E. coli gene. RGTT and LMPG are mini-project parts.

Stop Time: 8:00PM

Next: Retry PCR with Dr. Westenberg's primers for bacterial DNA (S16 Ribosome subunit)

Date: 03/18/14 People in lab: Levi Palmer, Caleb Trecazzi

Title: PCR of 16S ribosomal subunit from Genome prep, gel electrophoresis of products

Start Time: 2:00 PM

Purpose: To confirm usable DNA from the genome prep

Protocol: LTM ed. 2 **Exceptions:** 1. 2uL of each primer used, 2. No DNA and no primer controls. 3. Annealing temp: 50C 4. Elongation time: 3min

Well	1	2	3	4	5	6	7	8
Sample	Sans primer		S16		S16		Sans DNA	

Results: S16 bands present, controls absent.

Stop Time: 7:00 PM

Next: Retry norCB PCR with different temp

Date: 03/19/14 People in lab: Levi Palmer, Caleb Trecazzi

Title: PCR of hmp and TOPO-TA cloning with transformation

Start Time: 4:00 PM

Purpose: To amplify hmp gene from E. coli and put into TOPO vector to transform into E. coli(DH5alpha)

Protocol: LTM ed. 2, Invitrogen TOPO-TA cloning manual **Exceptions:** 1. 6min incubation time

Products:

Sample Label	Description	Source Label	Quantity
hmp TOPO 3/19 1	hmp in TOPO vector, plated 1uL PCR product used	E.coli 8/6/13	4
hmp TOPO 3/19 2	hmp in TOPO vector, plated 2.5uL PCR product used	E.coli 8/6/13	4
hmp TOPO 3/19 3	hmp in TOPO vector, plated 4uL PCR product used	E.coli 8/6/13	4

Results:

Notes: Variable amounts of PCR product used to see what works best.

Stop Time: 7:00 PM

Next: Blue/white colony screening to check for colonies with insert

Date: 03/20/14 People in lab: Levi Palmer, Caleb Trecazzi

Title: Gel electrophoresis of 3/19 PCR to check for presence of hmp gene product

Start Time: 2:00 PM

Purpose: To confirm presence of hmp gene in 3/19 PCR

Protocol: LTM ed. 2

Well	1	2	3	4	5	6	7	8
Sample								

Results:

Notes:

Stop Time:

Next: